



Received on 01 March 2014; received in revised form, 17 April 2014; accepted, 30 May 2014; published 01 September 2014

ASSESSMENT OF β -ADRENORECEPTOR ANTAGONISTIC ACTIVITY OF NS1 AND NS2 ON PERCENTAGE INHIBITION OF ISOPRENALINE INDUCED TACHYCARDIA AND HYPOTENSION IN RATS

Sanjay Kumar Gupta* and Rajesh Choudhary

Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur - 495009, Chhattisgarh, India.

Keywords:

β -adrenoreceptor,
Isoprenaline, Hypotension,
Tachycardia, NS1 and NS2

Correspondence to Author:

Sanjay Kumar Gupta

Institute of Pharmaceutical Sciences,
Guru Ghasidas Vishwavidyalaya,
Bilaspur - 495009, Chhattisgarh,
India.

E-mail: sanjaypharm03@gmail.com

ABSTRACT: The present investigation is carried out for β -adrenoreceptor antagonistic activity of both derivatives by evaluating their effects on the isoprenaline-induced tachycardia and hypotension in rats. Rats were divided into 5 groups (n=6) based on the treatment given to them. Group 1 received saline (0.9 % NaCl) only, groups 2, 3 were treated using test drugs NS1 and NS2 (1 mg/kg, i.v.) and groups 4, 5 received standard drugs propranolol (2 mg/kg, i.v.) and atenolol (1 mg/kg, i.v.). After 15 minutes of injection of drugs, the isoprenaline was injected, and the change in mean arterial pressure and heart rate were recorded. Administration of isoprenaline alone (0.3, 1 and 3 μ g/kg, i.v.) produced significant dose-dependent hypotension and tachycardia to the rats. Pretreatment with NS1 and NS2 produced significant ($p < 0.01$ and $p < 0.05$ respectively) inhibition of isoprenaline-induced hypotension on normal rats. However, isoprenaline-induced tachycardia was significantly ($p < 0.05$) reduced by NS1 pretreatment only. Propranolol showed significant ($p < 0.01$) inhibition of isoprenaline-induced tachycardia and hypotension, while atenolol was significant ($p < 0.01$) effective against inhibition of hypotension only. Results indicated that both derivatives, *i.e.* NS1 and NS2, have the potential to inhibit β receptors, but NS1 showed higher affinity to inhibit β_1 receptors as compared to NS2. Hence study suggests that NS1 might have higher potency in β_1 adrenoreceptor blocking activity.

INTRODUCTION: Hypertension is one of the most common cardiovascular disorder and the leading cause of morbidity and mortality and major risk factor of premature death worldwide. According to the WHO report, about 1.56 billion adults will be living with hypertension in 2025¹. The aim of antihypertensive therapy is to reduce the elevated blood pressure without adversely affecting the quality of life.

Selection of the drugs is based on the mechanism behind the induction of hypertension to the patients, and the other diseases associate with the high blood pressure as, myocardial infarction, angina, and heart failure, *etc.*² Hypertension cannot be adequately controlled by monotherapy, and thus, it requires two or more antihypertensive agents to normalize the blood pressure and overcome the side effects of monotherapy³. The principle of multidrug therapy can be achieved by either two or more single active drugs are administered simultaneously, or many active agents are combined in one molecule that is called a hybrid molecule. These hybrid molecules often consist of different pharmacophoric groups which are linked to each other via spacers, and they act on different sites⁴.

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.5(9).3758-62</p> <hr/> <p>This article can be accessed online on www.ijpsr.com</p> <hr/> <p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(9).3758-62</p>
---	--

Introduction of β -adrenoreceptor blockers produces a promising efficacy on various cardiovascular diseases in the last few years. β -adrenoreceptor blockers have also been established in the treatment of myocardial infarction, angina pectoris, and congestive heart failure. β -adrenoreceptor blockers also reduced the mortality and morbidity of hypertensive patients^{5, 6}. Recently, β -adrenoreceptor blockers are clinically used alone or in combination with various antihypertensive agents such as diuretics, angiotensin-converting enzyme inhibitors, and calcium channel blockers to treat various cardiovascular disorders such as hypertension, myocardial infarction and heart failure^{7, 8}. Being impressed by such recent trends, a superior vasodilator drug with the beta-blocking property was synthesized **Fig. 1**.

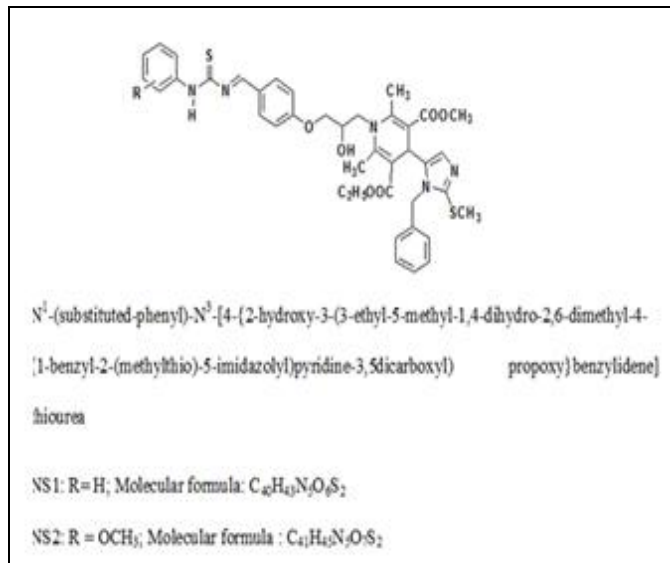


FIG. 1: BASIC CHEMICAL STRUCTURE OF THE SYNTHESIZED HYBRID NS SERIES COMPOUNDS

Synthesized hybrid molecules have a thiourea moiety, which is reported to possess vasodilator activity upon the structural modification and the other moiety is calcium channel blocker [3-methyl-5-ethyl - 1, 4 - dihydro - 2, 6-dimethyl - 4 - (1-phenylmethylthio-5-imidazolyl) pyridine - 3, 5-dicarboxylate] attached through beta-blocking moiety (propranolamine). The antihypertensive effects of both derivatives NS1 and NS2 were already reported in our previous study⁹. In the present investigation, we evaluated the β -receptor blocking potential of NS1 and NS2 by studying their effects on percentage inhibition of isoprenaline (Iso) induced hypotension and tachycardia in the experimental rats.

MATERIALS AND METHODS:

Animals: Wistar albino rats (180-220 g) of either sex were procured from AISSMS College of Pharmacy, Pune. Standard laboratory conditions of temperature 24 ± 2 °C, relative humidity $55 \pm 5\%$ and 12:12 h light dark cycle were maintained throughout all the experiments according to the guidelines of Committee for Control and Supervision of Experiments on Animals (CPCSEA). Rats were fed standard pellet animal diet (Chaken oil Mill, Pune; India) with water *ad libitum*. All the experimental procedures and protocols approved by the Institutional Animal Ethics Committee (IAEC) of AISSMS College of Pharmacy, Pune, constituted under CPCSEA. Proposal number: CPCSEA/IAEC/PC-03/09-2K7.

Drugs and Chemicals: Test compounds NS1 and NS2 were received as gift sample, synthesized at the Department of Pharmaceutical Chemistry, AISSMS College of Pharmacy, Pune. Atenolol was obtained as a pure drug sample from Acme Formulation Pvt. Ltd., Himachal Pradesh. Propranolol (Cilpar 40 mg tab., Cipla), Isoprenaline (Isoprin, 2 ml inj., Unichem Laboratory ltd., India) and nifedipine (Cardipine, Intas), Ketamine (Ketmin 50, Themis Medicare Ltd, Gujarat), urethane (Rajesh chemicals, Mumbai) were procured from the local market. Ethanol and heparin (Thromboparin inj, Charls Pharma Inc), sodium chloride, potassium chloride, calcium chloride, magnesium sulfate. $7H_2O$, potassium dihydrogen phosphate, glucose, and sodium bicarbonate of laboratory grade were purchased from local vendors.

Preparation of Drug solution: For intravenous injection drug solutions were prepared by dissolving in sterile saline solution (0.9% NaCl solution). Doses were selected based on acute toxicity study.

In-vivo Assessment of β -Adrenoreceptor Antagonistic Activity of NS1 and NS2:

Preparation of Animals: Rats were anesthetized by urethane (1.25-1.5 g/kg, i.p.) with prior administration of atropine (0.2 mg/kg, s.c.) to avoid the cardiopulmonary problem associated with urethane anesthesia. Anesthetized rats were fixed on its back over the surgical table. Body temperature was maintained at 37 °C throughout

the experiment. An incision was made over the skin of neck region and left jugular vein was identified and made free from connective tissues with the help of blunt forceps. Cephalad end (towards the brain) of the vein was permanently occluded with help of ligature. Using an iris scissor a small transverse cut in the vein was made. Polyethylene cannula (PE 50) filled with heparinized saline (100 IU/ml) was passed into the vein and ligated. The left jugular vein was cannulated for intravenous drug administration. The left carotid artery was isolated and exposed, and a polyethylene cannula (PE 50) filled with heparinized saline was inserted into the artery and ligated for direct measurement of mean arterial pressure (MAP) ^{10,11}.

Measurement of Mean Arterial Pressure:

Heparinised saline (100 IU/ml) was filled inside the knob of a pressure transducer (SS 13L) and in the polyethylene catheter (PE-50) to prevent blood clotting. Cannulated carotid artery was connected to a pressure transducer linked to four channel data acquisition system (BIOPAC System Inc, MP 35). The bulldog clamp was removed from the carotid artery to divert the blood pressure to the transducer, and the MAP was allowed to stabilize, and then blood pressure was recorded ¹².

Measurement of Heart Rate: ECGs of rats were recorded with the application of subcutaneous stainless steel needles electrodes to the flexor aspect of limbs of rat and connected with the SS2L electrode transducer linked with the Four Channel Data Acquisition System (BIOPAC System Inc, MP 35). Measurement of heart rate was done by analyzing the R-R interval of ECG recording. Saline (0.2 ml, i.v.) was administered, MAP and heart rate (HR) was allowed to stabilize. An equilibration period of 30 min was given, during this period; animals having mean arterial blood pressure fluctuation by more than 10% were discarded ¹³.

Evaluation procedure for β Adrenoreceptor Antagonistic Activity of NS1 and NS2: Dose-response curve (DRC) to Iso was constructed for an increase in HR (tachycardia) and fall in MAP (hypotension) after intravenous injection of 0.3, 1 and 3 $\mu\text{g}/\text{kg}$ of Iso. Next, a single dose of test drug was administered intravenously. Fifteen minutes later, further injections of Iso (1 $\mu\text{g}/\text{kg}$, i.v.) were

given, and the changes in HR and MAP were recorded ^{14,15}.

Data Calculations and analysis: Responses to Iso were calculated as the percentage decrease in MAP and percentage increase in HR. Percent decrease in MAP, the percent increase in HR, and the percentage inhibition of Iso induced tachycardia and hypotension were calculated using the formulas mentioned below.

$$\begin{aligned} \% \text{ decrease in MAP} &= 1 - \frac{\Delta \text{ MAP after drug treatment}}{\Delta \text{ MAP before drug treatment}} \times 100 \\ \% \text{ increase in HR} &= 1 - \frac{\Delta \text{ HR after drug treatment}}{\Delta \text{ HR before drug treatment}} \times 100 \\ \% \text{ inhibition} &= 1 - \frac{\text{Effect of Iso alone} - \text{effect of Iso with antagonist}}{\text{Effect of Iso alone}} \times 100 \end{aligned}$$

Where Δ MAP represents the change in mean arterial blood pressure and Δ HR represents the change in HR. All values are expressed as mean \pm S.E.M (n=6). The statistical significance of the difference between groups was determined using one-way analysis of variance (ANOVA) followed by Dunnett's test. Differences were considered statistically significant at $P < 0.05$.

RESULTS:

Effect of Isoprenaline Alone on MAP and HR of Rats: Administration of Iso (0.3, 1 and 3 $\mu\text{g}/\text{kg}$, i.v), produced dose-dependent fall in MAP (hypotension) and increased the heart rate (tachycardia) in a dose-dependent manner **Fig. 2**.

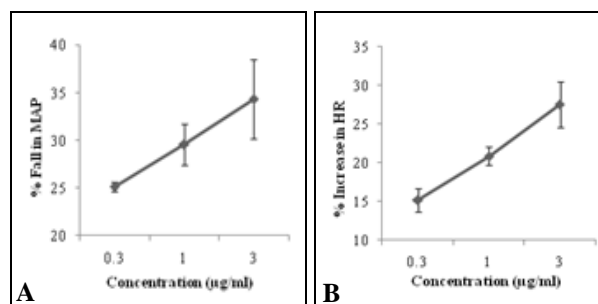


FIG. 2: CONCENTRATION RESPONSE CURVE OF ISO (0.3, 1 AND 3 $\mu\text{g}/\text{ml}$, I.V.) ON (A) MAP AND (B) HR

Hypotensive and Bradycardia Effect of NS1, NS2, Atenolol and Propranolol Alone in Rats: Intravenous injection of NS1 (1mg/kg, i.v.), NS2 (1 mg/kg, i.v.), atenolol (1mg/kg, i.v.) and propranolol (2 mg/kg, iv.) produced significant ($p < 0.01$) reduction in MAP and HR of rats as compared to saline-treated rats **Fig. 3**. However, NS1 was found

more effective than others in the reduction of MAP and HR.

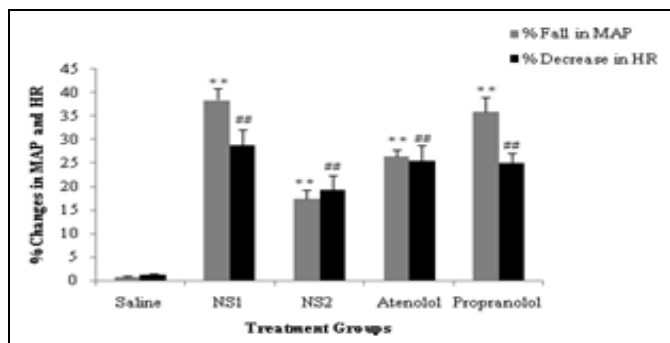


FIG. 3: EFFECT OF SALINE, NS1, NS2, ATENOLOL AND PROPRANOLOL ON THE MAP AND HR OF RATS AFTER I.V. ADMINISTRATION. Values are expressed as mean \pm S.E.M. (n = 6). ** p<0.01, ## p<0.01, significantly different compared with saline-treated rats.

Effect on Isoprenaline-Induced Hypotension and Tachycardia in Rats: Pretreatment with NS1 (1 mg/kg, i.v.) and NS2 (1 mg/kg, i.v.), both produced significant inhibition of Iso induced hypotension in normal rats; however, NS2 was less significant (p<0.05) than NS1 (p<0.01), whereas Iso induced tachycardia was significantly (p<0.05) reduced with NS1 pretreatment only. Reference standard propranolol showed significant (p<0.01) inhibition of Iso induced tachycardia and hypotension while atenolol was significant (p<0.01) effective against inhibition of hypotension only **Fig. 4**.

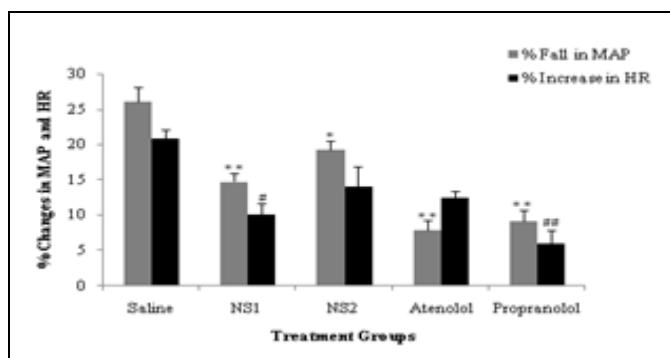


FIG. 4: EFFECT OF ISOPRENALINE (1 μ g/ml, i.v.) ON MAP AND HR AFTER I.V. ADMINISTRATION OF SALINE, NS1, NS2, ATENOLOL, AND PROPRANOLOL. Values are expressed as mean \pm S.E.M. (n = 6). * p<0.05, ** p<0.01, # p<0.05 ## p<0.01, significantly different compared with saline treated rats.

DISCUSSION: The synthesized hybrid molecules NS1 and NS2 are having three antihypertensive nucleuses, one with β -blocker activity, second with calcium channel blocker activity and third with vasodilator activity⁹. Though vasodilation reduces the blood pressure by directly relaxing peripheral vascular smooth muscles, the reduction leads to a

reflex increase in sympathetic tone followed by an increase in heart rate, cardiac output and plasma renin activity which attenuates antihypertensive effect. It was reported that this undesirable effect of vasodilator could be eliminated or minimized by simultaneous treatment with β -blocker and the possible increase in peripheral vascular resistance induced by β blocker was eliminated or minimized by concomitant use of vasodilators⁷.

Dihydropyridines (DHPs) cause tachycardia which is counteracted by β -blockers, while the initial increase in total peripheral resistance caused by non-selective β -blockers is counteracted by the vasodilators¹⁶. In the previous study, antihypertensive activity was evaluated using renal artery ligation, *i.e.* 2K1C (two kidneys one clip) model of hypertension, both NS1 and NS2 indicated an antihypertensive effect on renovascular hypertension. But since NS2 didn't affect the heart rate, it may be due to its less or non β_1 adrenoreceptor antagonistic activity⁹. The present study was carried out to determine in the *vivo* β -adrenoreceptor antagonistic effect of NS1 and NS2 by measuring their inhibitory effect on the Iso induced hypotension and tachycardia to the rats.

Iso is a potent non-selective β -adrenoreceptors agonist with high affinity for all β adrenoreceptors and low affinity for α -adrenoreceptors. Intravenous infusion of Iso stimulates cardiac β -adrenoreceptors and lowers peripheral vascular resistance.

The tachycardia produced by Iso is primarily due to the β_1 -adrenoreceptors activity in the heart and the hypotensive effect is primarily due to the β_2 -adrenoreceptors in the blood vessels. β -adrenoreceptor blockers prevent the hypotensive and tachycardia effect induced by Iso^{8,17}.

Our results indicated that administration of Iso alone produced significant dose-dependent hypotension and tachycardia in anesthetized rats **Fig. 2**. The percentage increase in heart rate and hypotension by Iso are in good correlation with earlier reports. NS1, atenolol, and propranolol followed by Iso injection in respective animals inhibited the Iso induced hypotensive effect greater than NS2. However, only NS1 and propranolol further produced significant (p<0.05 and p<0.01, respectively) inhibition of Iso induced tachycardia.

As propranolol is the competitive β -adrenoreceptor antagonist while atenolol is noncompetitive, due to this atenolol may not competes with Iso for β -adrenoreceptor and hence not significant inhibition heart rate that was increased by Iso.

CONCLUSION: This finding shows the competitive nature of NS1 towards β -adrenoreceptor, on the other hand, NS2, only inhibited the hypotensive effect of Iso, showing its β -adrenoreceptor antagonistic activity but being non-competitive it has not decreased the heart rate significantly. Hence, study suggests that NS1 might have higher potency in β -adrenoreceptor blocking activity than NS2 with more specificity to the β -1 receptor. Our finding in the study concluded that both NS1 and NS2 have a β -adrenoreceptor antagonistic effect, but NS1 found more effective than NS2. Further investigations of NS1 and NS2 on β -1 receptor antagonistic effects are under process.

ACKNOWLEDGEMENT: The authors are grateful to Acme Formulation Pvt. Ltd, Himachal Pradesh for providing gift sample of atenolol and Dr. K.G. Bothara, Principal, AISSMS College of Pharmacy for providing necessary support and guidance.

CONFLICT OF INTEREST: Nil

REFERENCES:

- World Health Organization. Regional Office for Southeast Asia. Hypertension fact sheet. 2011. (Accessed 24 December 2013) Available from: URL:http://www.searo.who.int/entity/noncommunicable_diseases/media/non_communicable_diseases_hypertension_fs.pdf.
- Gradman AH, Basile JN, Carter BL and Bakris GL: Combination therapy in hypertension. *Journal of the American Society of Hypertension* 2010; 4: 90-98.
- ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group: Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs. diuretic: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *Journal of the American Medical Association* 2002; 288: 2981-97.
- Christians JAM and Timmerman H: Cardiovascular hybrid drug: Combination of more than one pharmacological property in one single molecule. *European Journal of Pharmaceutical Sciences* 1996; 4: 1-22.
- Messerli FH, Bangalore S, Yao SS and Steinberg JS: Cardioprotection with beta-blockers: Myths, facts and Pascal's wager. *Journal of Internal Medicine* 2009; 266: 232-41.
- Bhatt LK, Bansal J, Piplani P, Bodhankar SL and Veeranjanyulu A: Cardioprotective and β -adrenoreceptor antagonistic activity of a newly synthesized aryloxypropranolamine derivative PP-36. *The Journal of Experimental Biology* 2010; 2: 37-45.
- Hoffman BB: Therapy of Hypertension. In: Handman JG, Limberd LE and Gilman AG (Eds): Goodman and Gilman's *The Pharmacological Basis of Therapeutics*. McGraw Hill, New York, Edition 10, 2001: 547-58.
- Rang HP, Dale MM, Ritter JM and Flower RJ: Rang and Dale's *Pharmacology*. Churchill Livingstone Elsevier, Six Edition 2007.
- Gupta SK, Vyawahare NS, Kagathara VG, Patil MN and Pujari RR: Assessment of antihypertensive activity of the novel synthetic hybrid compound on 2K1C induced hypertensive rats. *Pharmacologyonline* 2009; 2: 273-80.
- Bartosova L, Frydrych M, Hulakova G, Berankova K, Strnadova V, Morky P, Brunclik V, Kolevska J and Bebarova M: Effect of newly synthesized 44Bu ultrashort-acting Beta-adrenergic antagonist to isoprenaline-induced tachycardia comparison with esmolol. *Acta Vet Brno* 2004; 73: 171-79.
- Nandakumar K, Singh R, Bansal SK, Bodhankar SL, Jindal DP, Coumar MS and Bhardwaj SH: The antihypertensive effect of newly synthesized acyl amino substituted propranolamine derivatives, DPJ 890 and DPJ 955 in rats. *Indian Journal of Pharmacol* 2004; 36: 381-84.
- Nandakumar K, Bansal S, Singh R, Mohite A, Bodhankar SL, Jindal DP, Coumare M, Balaraman R and Bharadwaj SH: Study of β -adrenoreceptor antagonistic activity of DPJ 904 in rats. *Pharmacology* 2005; 74: 1-5.
- Kharin SN, Krandycheva VV and Shmakov DN: Depolarization pattern of the ventricular epicardium in two-kidney one-clip hypertensive rats. *Experimental Physiology* 2005; 90: 621-26.
- Nandakumar K, Bansal SK, Singh R, Bodhankar SL, Jindal DP, Coumar MS, Balaraman R, and Bhardwaj SH: Selective β -adrenoreceptor blocking the activity of newly synthesized acyl amino-substituted aryloxypropranolamine derivatives DPJ 955 and DPJ 890, in rats. *Journal of Pharmacy and Pharmacology* 2005; 57: 515-20.
- Dube A, Choudhary S, Bodhankar SL and Piplani P: Study of beta-adrenoreceptor blocking and antihypertensive activity of a newly synthesized aryloxypropranolamine derivative, pp-28 in laboratory animals. *Pharmacology online* 2010; 1: 770-82.
- Tripathi KD: *Essential of medical pharmacology*. Jaypee Brothers, Six Edition 2006.
- Chiu CC, Lin YT, Tsai CH, Liang JC, Chiang LC, Wu JR, Chen IJ and Yeh JL: Pharmacological effects of an aldehyde type α/β adrenoreceptor-blocking agent with vasodilating properties. *General Pharmacology* 2000; 34: 391-00.

How to cite this article:

Gupta SK and Choudhary R: Assessment of β -adrenoreceptor antagonistic activity of NS1 and NS2 on percentage inhibition of isoprenaline induced tachycardia and hypotension in rats. *Int J Pharm Sci & Res* 2014; 5(9): 3758-62. doi: 10.13040/IJPSR.0975-8232.5(9).3758-62.